EXHIBIT J

of the tissue, reminiscent of QIR formation (Fig. 2).

Conclusions: Engineered human bladder models could be used as a replicable test bed for chronic infective disease formation, treatment and resolution in humans.

References: 1. American Journal of Clinical Pathology, 2006. 125(1): p. 105–110.
2. Annu Rev Microbiol, 2010. 64: p. 203–21.
3. PLoS One, 2013. 8(12): p. e83637.

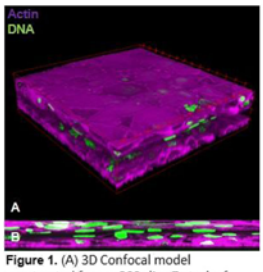

Figure 1. (A) 3D Confocal model constructed from a 200 slice Z-stack of engineered human bladder tissue. Umbrella cells are large and flat and frequently bi-nucleated. (B) Orthogonal view of Z-stack shows the tissue to be ~5 layers in depth and the basal cells to be spheroid in morphology.

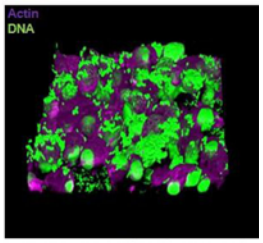

Figure 2. 3D Confocal model constructed from a 200 slice Z-stack of engineered human bladder tissue post infection with *E. faecalis*. Large biofilm-like clusters of bacteria (green) can be seen adhering to the luminal surface of intermediate and basal urothelial cells (magenta).

Disclosure Block:

Harry Horsley: Nothing to disclose. James Malone-Lee: Pfizer: Speaker, Honoraria. Astellas: Speaker, Honoraria. Jennifer Rohn: Nothing to disclose.

PP 29

OXIDATIVE DEGRADATION OF POLYPROPYLENE PELVIC MESH IN VITRO

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Abstract:

Introduction: Surgical treatment options for stress urinary incontinence and pelvic organ prolapse include reconstruction of connective tissue with biological or synthetic grafts. The most common synthetic grafts are non-resorbable stabilized polypropylene (PP) meshes. However, complications arising from mesh implantation can lead to graft erosion and bladder outlet obstruction (1). In PP meshes explanted following graft complication, histological and chemical analysis revealed infection or chronic inflammation as well as evidence of PP degradation (2). The ex vivo oxidative degradation mechanism of PP proceeds through a stable hydroperoxide (−COOH) intermediate prior to chain scission and the formation of a carbonyl (−C=O) end group. Since the force applied to mid-urethral slings is in the range of 5–15 N, we reasoned that the combination of mechanical stress, the secretion of reactive oxygen species (ROS) by adherent inflammatory cells associated with the foreign body reaction, and the susceptibility of PP to oxidation leads to environmental stress cracking as a potential root cause of failure of PP pelvic mesh in patients.

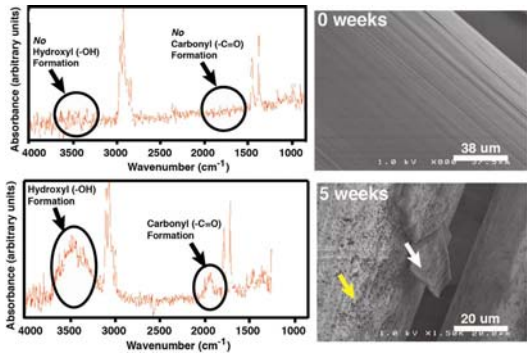
Objective: In the present study, we investigated oxidative degradation of PP meshes in vitro using an oxidative medium that recapitulates the microenvironment between an adherent macrophage and the PP surface.

Methods: Pelvic PP mesh (stabilized by antioxidants added during manufacture) and unstabilized PP pellets (control) were incubated at 37 °C for up to 5 weeks in oxidative media composed of 0.1 M CoCl2 in 20 wt.% H2O2, which simulates the microenvironment between an adherent macrophage and the biomaterial surface (3). Every week 6 samples were removed, washed in DI water, and dried for analysis. Outcomes were assessed by x-ray photoelectron spectroscopy (XPS, n=3) and Fourier Transform Infrared Spectroscopy (FTIR, n=3) to test for the presence of hydroxyl (−OH) groups present in the hydroperoxide intermediate and for terminal -C=O end groups. SEM images were taken at 0, 4, and 5 weeks to identify evidence of oxidative degradation, such as pitting, flaking, and cracking. The degradation of the stabilized PP mesh samples was compared to that of unstabilized PP pellets tested following the same protocol. In all cases, oxidative media was changed every 3–4 days (3).

Results: FTIR spectra taken after 0 weeks immersion time showed no -OH or -C=O peaks for either unstabilized PP pellets (not shown) or PP pelvic mesh (top left panel). However, after 5 weeks incubation time, FTIR spectra showed the appearance of both -OH and -C=O groups, which indicates oxidative degradation (bottom left panel). XPS analysis also revealed evidence of -OH and -C=O groups on the surface. The dramatic increase in the size of the -OH and C=O peaks from 4 (not shown) to 5 weeks is indicative of chemical induction. FTIR spectra of unstabilized PP pellets showed significant -OH and -C=O peaks at 4 weeks. Thus, the presence of antioxidants in the PP pelvic mesh delayed induction for a period of time <30 % longer than that observed for unstabilized PP. SEM images at 0 weeks showed longitudinal features presumably resulting from extrusion during manufacture (top right panel). No evidence of surface degradation was observed. However, SEM images of PP pelvic mesh incubated for 5 weeks in oxidative media (lower right panel) revealed evidence of pitting (yellow arrow) and flaking (white arrow).

Conclusions: Oxidative degradation of PP pelvic mesh was evidenced by chemical and physical changes under simulated in vivo conditions. These data underscore the need for further research into the relative contribution of oxidative degradation to the failure of PP-based pelvic organ prolapse and stress urinary incontinence devices.

References: 1. BJOG 2008;115:1350–1361. 2. Int. Urogynecol. J. 2010;21:261–270. 3. J Biomed Mater Res 1997; 34:519–530



Disclosure Block:

Scott Guelcher: Polymer & Chemical Technologies: Consultant, Consulting Fee. Guelcher Consulting: Consultant, Ownership Interest. Russell Dunn: Nothing to disclose.

PP 30

CANNABINOID RECEPTOR EXPRESSION IN THE BLADDER IS ALTERED IN DETRUSOR OVERACTIVITY

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Abstract:

Background: We have previously shown that cannabinoid agonists inhibit rat detrusor muscle contractility. This study compares cannabinoid receptor (CBR) expression in normal human bladders and in bladders with detrusor overactivity (DO). Immunohistochemical evidence shows that CBR are expressed in human bladders and cannabinoid agonists are known to inhibit detrusor muscle contractility.

Objective: The aim of this study was to compare differences in CBR expression and distribution in patients with DO and normal bladders and further characterize these receptors by co-localization studies with nerve markers. In addition, saturation binding experiments in human bladder, rat bladder and cerebellum were performed to characterize CBR and evaluate affinity (K_d) and receptor density (B_{max}).

Methods: Quantitative PCR was used to detect differences in CB1 and CB2 receptor transcripts in bladder samples. Differences in CBR protein expression were assessed by immunohistochemistry (IHC). Double staining immunofluorescence (IF) was used to evaluate co-localisation of CBR with nerve fibres. K_d and B_{max} was measured using the non-selective cannabinoid agonist [^{3}H]-CP55,940.

Results

Higher levels of CB1 receptor mRNA expression was found in the urothelium of patients with DO compared to normal urothelial samples (p=0.002). In contrast, patients with DO had lower levels of CB1 receptor in the detrusor muscle compared to normal detrusor samples (p=0.0012). Mean±SEM of the different groups are shown in **Table 1.**

Table 1. PCR transcripts in human bladder

Receptor	Normal Urothelium (n=5)		DO Urothelium (n=4)	
	Average Ct values ±SEM	ΔCt relative to GAPDH	Average Ct values ±SEM	ΔCt relative to GAPDH
CB1	35.1±0.5	8.4±0.3	31.7±0.4	6.9±0.5
CB2	36.4 ±0.4	10.7±1.0	33.6±0.5	9.1±1.9
	Normal Detrusor (n=5)		DO Detrusor (n=4)	
CB1	30.6±0.6	6.8±0.4	34.6±0.7	8.5±0.7
CB2	33.5±0.4	10.4±0.4	37.0±1.0	11.4±1.0

Radioligand binding analysis revealed total CB receptor expression of 421±104fmol/mg protein in normal human bladders (n=5). See **Table 2** for K_d and B_{max} for different types of tissue studied. IHC confirmed these findings at the protein level (**Figs. 1 & 2**). Double IF staining showed co-localisation of CB1 with Choline Acetyltransferase (ChAT) positive nerves in detrusor and co-localisation with PGP9.5 in both urothelium and detrusor. CB2 was co-localised with both ChAT and PGP9.5 in urothelium and detrusor.

Table 2. Saturation-binding assays. Receptor density (B_{max}) is expressed as the amount of protein per membrane unit (fmol mg^{-1}). Data are mean ± SEM for ≥ 5 experiments.

	pK_d (nM)	B_{max}
Human bladder	8.9 ± 0.2 (1.26)	421.4 ± 104.2
Rat Bladder	9.4 ± 0.1 (0.39)	429.7 ± 102.1
Rat cerebellum	9.3 ± 0.1 (0.45)	1974 ± 308.2

Conclusions

CBR expression is reduced in patients with DO, which may contribute to the increased contractility seen in these patients. Binding affinity of CBR using CP55,940 has not been measured before in human bladder and was found to be lower than reported in brain but higher